Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

SARS-CoV-2 adaptive immunity in nursing home residents up to eight months after two doses of the Comirnaty® COVID-19 vaccine

Dear Editor,

There is scant information as to how SARS-CoV-2 antibody and T-cell immune responses elicited by mRNA COVID-19 vaccines evolve in the general population, and in particular in elderly nursing home residents, who are at increased risk of developing severe clinical forms of COVID-19. We read with interest the work by Tré-Hardy and colleagues who reported a significant antibody decrease at around 6 months after full vaccination in healthcare workers, that was more marked in SARS-CoV-2 naïve vaccinees [1]. The authors suggested that in a supply-limited environment, booster dose schemes may be spared for SARS-CoV-2-experienced individuals. The data presented herein extend this observation to elderly nursing home residents. The current prospective cohort study included 680 (478 female; median age, 87 years; range 65–100) of a cohort of 881 nursing home residents initially recruited from a representative sample of Valencian Community nursing homes ($n = 13$) for assessment of SARS-CoV-2 immune responses at a median of 3 months (3 M) following full-dose Comirnaty® COVID-19 vaccination [2] who were re-examined at a median of 219 days (range, 139–246) after vaccination (7 M). Out of the 680 participants, 238 had been infected by SARS-CoV-2 prior to receiving the first vaccine dose, as recorded in the electronic Valencia Health System Integrated Databases. Two residents contracted the infection (Delta variant, as documented by whole-genome sequencing) between sampling times (3 M and 7 M). The remaining 440 participants were presumably naïve for SARS-CoV-2 at the time of sampling (7 M).

The current study was carried out under the epidemiological surveillance competences of the Valencia Government Health Department (Law 16/2003/May 28 on Cohesion and Quality of the National Health System, and Law 10/2014/ December 29 on Public Health of the Valencian Community), without requiring informed consent or ethics approval by an institutional review board. Likewise, in accordance with local law and regulations, data publication is exempt from the research ethics committee approval. Personal data from nursing homes and residents were processed in accordance with European data protection regulations.

All participants were initially examined for presence SARS-CoV-2-Spike (*S*)-specific antibodies in whole blood obtained by fingerstick using a lateral flow immunochromatographic assay (LFIC): the OnSite COVID-19 IgG/IgM Rapid Test (CTK BIOTECH, Poway, CA, USA) [2]. As shown in Table 1, a total of 148 of the 680 (21.7%) residents tested negative by LFIC at 7 M. The percentage of residents without detectable anti-S LFIC responses at 7 M was roughly double the proportion at 3 M. Moreover, overall, the strength of antibody reactivity [2] in LFIC among those who tested positive at both sampling times ($n = 520$) tended to decrease by 7M: 169, 84 and 267 residents showed decreased, increased or similar antibody reactivity grades, respectively. Interestingly, SARS-CoV-2-experienced participants were more likely to display detectable and higher grade antibody responses at 7 M than SARS-CoV-2-naïve participants (Fig. 1A). Indeed, negative LFIC results were registered in 11/238 (4.6%) and 137/440 (31%) of SARS-CoV-2-recovered and naïve residents, respectively ($P<0.001$; Fisher exact test), while antibody reactivities grade ≥2 were present in 181/238 (76%) and 118/440 (26.8%) of SARS-CoV-2-experienced and naïve residents, respectively ($P<0.001$).

Participants testing negative by LFIC underwent for quantitation of receptor binding domain (RBD)-reactive total antibodies using an (Electro)chemiluminescent –(E)CLIA- immunoassay (Roche Elecsys® Anti-SARS-CoV-2-S, Roche Diagnostics, Pleasanton, CA, USA), and IgG antibodies against a trimeric S-protein antigen by employing CLIA (LIAISON® SARS-CoV-2 TrimericS IgG assay; DiaSorin S.p.A, Saluggia, Italy) in plasma. Antibody testing could be performed in 144 of the 148 residents, of which 138 (95.8%) tested positive by RBD ECLIA and 108 (75%) by S-trimeric assay. Taking the above data together, 670/676 residents undergoing testing by LFIC and (E)CLIA (99.1%) exhibited detectable S-reactive antibody responses by 7 M, a similar figure (98%) to that reported in the original cohort at 3 M after vaccination [2].

A total of 100 residents had 3 M/7 M paired plasma specimens analyzed by RBD ECLIA. As shown in Fig. 1B, overall antibody levels declined over time, but particularly at the expense of SARS-CoV-2-naïve participants.

Participants testing negative for SARS-CoV-2 antibodies by all the above assays ($n = 6$) with available specimens ($n = 5$) were examined for presence of SARS-CoV-2-S-reactive IFNγ-producing T cells by whole-blood flow cytometry for intracellular cytokine staining (ICS), as previously described [2,3]. Four residents had detectable S-targeted $CD8^+$ T cells (median, 0.47%; range, 0.16–3.94%), whereas none had $CD4^+$ T cells. We next examined 28 randomly selected participants (25 SARS-CoV-2-naïve and 3 experienced) testing negative by LFIC but positive by (E)CLIA: 23 displayed detectable $CD8^+$ T-cell responses (median, 0.24%; range, 0.01–2.88%), 3 had both $CD8^+$ and $CD4^+$ T-cell (median, 0.44%; range, 0.03–0.77%) responses and 2 had neither.

Paired 3 M/7 M whole-blood specimens were available from 24 residents (Supplementary Table 1). Examining SARS-CoV-2-S-reactive IFNγ-producing $CD8^+$ T cells, we observed that 8 residents who had not detectable responses at 3 M acquired them by 7 M, whereas 16 had documented responses at 3 M, which were maintained in 14 and lost in 2. Regarding SARS-CoV-2-S-reactive IFNγ-producing-$CD4^+$ T cells, most responders at 3 M (21/22) no longer had detectable responses at 7 M, whereas 1 out of 2 residents acquired them by 7 M Fig. 1.C illustrates that while SARS-CoV-2-S-reactive IFNγ-producing $CD8^+$ T-cell levels in-

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Table 1
Anti-SARS-CoV-2-Spike (S) antibody reactivity as determined by lateral flow immunochromatography in nursing home residents by 3 and 6 months after full-dose vaccination with the Comirnaty® COVID-19 vaccine.

Anti-S antibody reactivity by 3 months (median) after full-dose vaccination	Anti-S antibody reactivity by 7 months (median) after full-dose vaccination (number of residents)			
	0	1+	2+	3+
0	73	8	2	2
1+	57	119	35	6
2+	15	90	101	43
3+	3	15	64	47

The IgG line intensity was scored visually using a 4-level scale, as previously reported [3]: 0 = negative result; 1+ = intensity of test band lower than control band; 2+= intensity of test band equal to control line; 3+ = intensity of test band greater than control line. Reactivities ≥2 in the LFIC assay corresponded roughly to antibody levels ≥250 IU/ml as measured by Elecsys® Anti-SARS-CoV-2 S-total antibody assay (Roche Diagnostics, Pleasanton, CA, USA).

Supplementary Table 1
SARS-CoV-2-S reactive antibodies and T-cells in nursing home residents with paired specimens collected at 3 and 7 months after full Comirnaty® COVID-19 vaccination.

Patient code[a]	Sex	SARS-CoV-2 infection status	Anti-SARS-CoV-2 RBD Antibody level (IU/ml)[b]		SARS-CoV-2-S-reactive IFN-γ-producing T cells (3 M)		SARS-CoV-2-S-reactive IFN-γ producing-T cells (7 M)	
			3M	7M	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)
1	F	Recovered (infection acquired 90 days prior the first vaccine dose	30	16	1.07	0.62	ND	0.11
2	F	Naïve	ND	ND	ND	ND	ND	ND
3	M	Naïve	ND	6.9	ND	ND	0.03	1.26
4	F	Naïve	11.9	2	2.10	0.85	0.44	0.80
5	F	Naïve	9.68	10	1.88	0.48	ND	0.87
6	F	Naïve	51.6	39	0.06	0.02	ND	1.43
7	M	Naïve	49.9	22.4	0.47	0.40	ND	0.13
8	F	Naïve	ND	ND	0.02	ND	ND	3.94
9	M	Naïve	11.7	5.9	0.03	ND	ND	1.08
10	F	Naïve	17.5	16	0.10	ND	ND	0.54
11	F	Naïve	97.4	82	0.54	0.34	ND	0.16
12	F	Naïve	54.8	65	1.17	0.15	ND	0.04
13	F	Naïve	32.3	23	0.69	0.37	ND	0.62
14	F	Naïve	53	36	0.51	0.11	ND	2.88
15	F	Naïve	25.3	28	1.27	0.67	ND	0.01
16	F	Naïve	168	142	0.41	ND	ND	0.19
17	M	Naïve	58.6	47	0.08	ND	ND	0.28
18	F	Naïve	81	29	0.03	ND	ND	0.08
19	F	Naïve	120.7	48	0.45	0.08	ND	0.90
20	F	Naïve	49.1	33.6	0.90	0.08	ND	0.05
21	M	Naïve	1.9	2.8	1.67	0.81	ND	ND
22	M	Naïve	12.4	11.5	0.47	0.28	ND	ND
23	M	Naïve	15.8	20	2.68	0.33	ND	0.09
24	M	Naïve	35.5	31	1.30	0.61	ND	0.50

3 M, a median of 3 months after full-dose vaccination; 7 M, median of 7 months after full-dose vaccination; ND, not detectable; RBD, receptor binding domain of Spike (S) protein.
[a] Paired 3 M/7 M whole-blood specimens were available from 24 residents (23 SARS-CoV-2-naïve and 1 recovered).
[b] Elecsys® Anti-SARS-CoV-2 S-total antibody assay (Roche Diagnostics, Pleasanton, CA, USA).

creased slightly over time ($P = 0.12$), those of $CD4^+$ T cells declined dramatically ($P<0.001$). That most residents maintained detectable S-targeted $CD8^+$ T-cell responses at 7 M was in contrast to previously published data [4] reporting positive SARS-CoV-2 T-cell responses as determined by the QuantiFERON assay in only 5% of SARS-CoV-2-naïve participants at 24 weeks after full vaccination with the Comirnaty® vaccine. Nevertheless, it is uncertain how SARS-CoV-2 QuantiFERON assay and our flow cytometry ICS method compare analytically.

Limitations of the current study included the use of a semi-quantitative LFIC for front-line antibody testing and that functional specificities of SARS-CoV-2-S-reactive T cells beyond IFN-γ production were not explored.

In conclusion, our data indicated that both antibody and peripheral blood $CD4^+$ T-cell levels measured after Comirnaty® vaccination in elderly nursing home residents wane over time in line with previous findings [2–8], declining significantly by 7 M after vaccination, particularly in SARS-CoV-2 naïve individuals.

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CONFLICTS of INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

EG, EA, JSB, SP, DS, HV, RL, MJA, JS-P, JD, IC and FG-C: Methodology and data validation. JSB, SP, and DN: Conceptualization and data analysis. DN: writing the original draft. All authors reviewed the original draft.

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By March 2021, the Valencian Community had set up a COVID-19 vaccine research program (ProVaVac) (Decree 10/2021 of 16

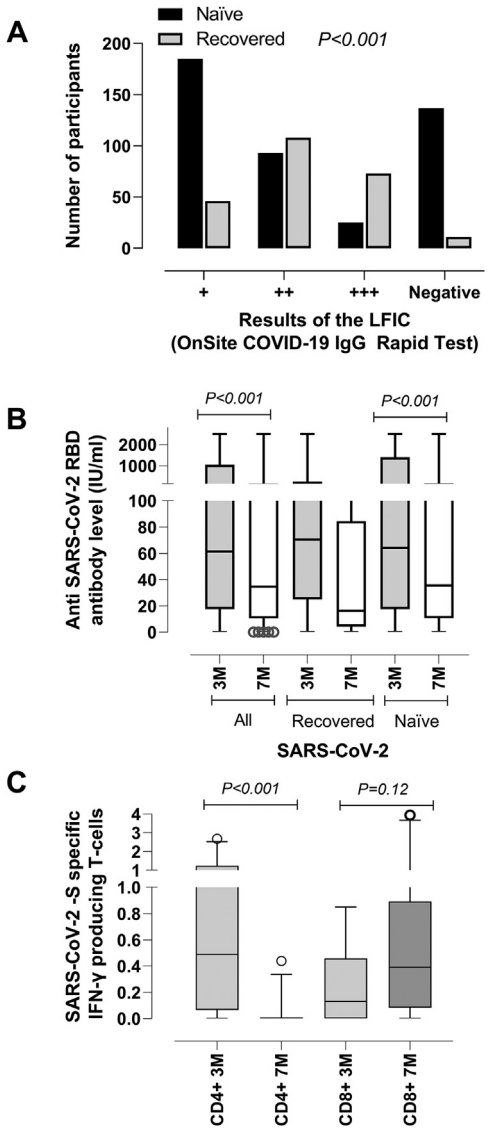

Fig. 1. SARS-CoV-2 antibody and T-cell immunity in nursing home residents up to eight months after two doses of the Comirnaty® COVID-19 vaccine. (A) SARS-CoV-2-Spike IgG reactivity of plasma from SARS-CoV-2-naïve and experienced nursing home residents at a median of 7 months after full vaccination with the Comirnaty® vaccine, as determined by the OnSite COVID-19 IgG/IgM Rapid immunochromatography Test (CTK BIOTECH, Poway, CA, USA). The IgG line intensity was scored visually using a 4-level scale as previously reported: 0 = negative result; 1+ = intensity of test band lower than control band; 2+ = intensity of test band equal to control line; 3+ = intensity of test band greater than control line. (B) SARS-CoV-2-Spike total antibody levels as measured by Roche Elecsys® assay (Roche Diagnostics, Pleasanton, CA, USA) in paired plasma specimens collected from 100 either SARS-CoV-2-naïve or -experienced nursing home residents at a median of 3 months (3 M) and 7 months (7 M) after full Comirnaty® vaccination. Both assays are calibrated to the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody [9] and provide quantitative values that strongly correlate with SARS-CoV-2 neutralizing antibody titers [10]. (C) SARS-CoV-2-Spike-reactive IFNγ-producing CD4$^+$ and CD8$^+$ T cells, as enumerated by flow cytometry for intracellular staining in paired whole-blood specimens collected from 24 nursing home residents at a median of 3 months (3 M) and 7 months (7 M) after full Comirnaty® vaccination. Differences between medians were compared using the Mann–Whitney *U test* or the Wilcoxon test, as appropriate. Two-sided exact *P*-values are reported. A *P*-value <0.05 was considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

March) which among other assignments was tasked with evaluating the immunogenicity of SARS-CoV-2 mRNA vaccines among nursing home residents. We are grateful to the Vice-presidency and Ministry of Equality and Inclusive Policies of the Valencia Community, the Corporate Association of Residences and Services for People with Dependency of the Valencian Community (AERTE), the Valencia Health System nursing home departmental committees, and the staff and residents of the participating nursing homes for their collaboration in developing the ProVaVac program. We are also grateful to Ana Berenguer, General Director of Analysis and Public Policies of the Presidency of the Generalitat. Eliseo Albert (Juan Rodés Contract; JR20/00011) and Estela Giménez (Juan Rodés Contract, JR18/00053) hold contracts funded by the Health Institute Carlos III (co-financed by the European Regional Development Fund, ERDF/FEDER).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.02.035.

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